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Tracking performance in elite athletes

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Abstract. There is a growing trend towards a personalized approach of professional athletes when it comes to health monitoring. Of particular interest was metabolic monitoring and therefore leading the training program and the diet of elite athletes with science. The aim of this paper is to present a review of the literature regarding the monitoring of biomarkers in elite athletes and the ways it can influence sports performance. Recent studies of exercise physiology related biomarkers have enabled a more comprehensive characterization of how to improve the effectiveness of training and prevent injuries based on the metabolic signature of athletes. The evaluation of athletes is an ongoing process. Each athlete has its own optimal range of biomarkers. When it comes to precisely assess the performance of athletes, researchers draw attention to the fact that a single biomarker measurement does not provide accuracy. Tracking athletes requires an integrative and dynamic approach. Several measurements will allow sports medicine clinicians to personalize training and diet programs based on each athletes' results. It is suggested to test athletes during off season in order to establish their own range, then, during the pre-season to evaluate their general health before competition, during competition and several times after that to assess the potential of recovery. Studies have indicated that regular monitoring of team and individuals sports athletes and also individuals within a team can identify inadequate recovery, nutritional deficiencies, inappropriate hormonal and immunological responses, assess bone and muscle health, monitor the electrolytes balance, track cognitive function and, finally, optimize match play-peak performance. A better understanding of the dynamic metabolic changes could result in an early diagnosis of Overtraining Syndrome. The trends of biomarkers could be integrated, in future perspective, in the periodic health evaluation of athletes with a great importance in the research related to the biological passport.
Key words: *biomarkers, overtraining, muscle status, performance.*

Introduction

The volume of training undoubtedly places a significant physiological and psychological stress upon the individual concerned. A critical threat to athletic performance is represented by the loss of training through either illness or injury. Monitoring the training load during training and competition is a routine in elite sports. Many different methods are used nowadays as part of the scientific monitoring and testing of the competitive athlete. Among them, physiological, biomechanical and movement assessments, GPS-based tracking systems, heart rate monitors, power meters, and training software are an integrative part of the scientific monitor program of many teams and athletes (1). However, there can be interpretation bias while assessing the internal and external load and also it is difficult to identify the causative mechanism. In order to fill this gap, biomarkers started to be used in monitoring the athlete in an integrative and dynamic process. Through testing and tracking the key biomarkers of athletes, it is possible to gain valuable information into whether they are overtraining, at increased risk of injury or if their body is fatigued. This will not only help physicians to optimize athletes' performance but also help to protect and improve their health.

A sustainable performance is built on a healthy life and in this way laboratory diagnostics represent an important diagnostic pillar in the medical care of competitive athletes. With the help of suitable laboratory parameters, a lot of information about the health of athletes can be obtained. Furthermore, laboratory diagnostics are important both in the prevention as well as in the diagnosis and rehabilitation of injuries. Taking into account the increasing training and competition loads in elite athletes, prevention is of great importance (2).

The aim of this work is to provide an overview of practice-relevant biomarkers, which should provide relevant information for the medical top athletes. For an analyte to be considered as a prognostic, diagnostic, and acceptable marker, it has to go through five stages of evaluation: analytic (precision and accuracy),

diagnostic (sensitivity and specificity), patient outcome efficacy (medical decision making), operational (predictive value and efficiency) and cost/benefit (societal efficacy) (3).
Exercise physiology research has identified individual biomarkers for assessing health, performance, and recovery during exercise training. Lee et al. recommended a panel of biomarkers in key categories as follows: nutrition and metabolic health, hydration status, muscle status, endurance performance, injury status and risk, and inflammation (4). Metabolomics represent a more comprehensive and detailed approach for detecting metabolic changes in response to dietary, lifestyle and environmental factors including profiles associated with performance, fatigue, and health issues (5).
However, biomarker testing poses many challenges because single biomarkers are not definitive for diagnosing broad physiological function and their sensitivity to detect overtraining or injury risk is limited, reference values for athletes well defined and interindividual variability exists. A single measurement of a biomarker does not allow for precise determination of an individual's health status (4).

Timing of testing
When making a schedule of monitoring various biomarkers, physicians have to take into consideration that the absolute resting levels of biomarkers may not change, but the response to stress could be abnormal. Thus, each athlete's average resting levels measured over multiple days are relevant to interpretation. Also, it is of great importance to understand the normal fluctuations in biomarkers in response to training and recovery for a given athlete over a period of time.
Lee et al. suggest testing before and at the end of preseason training in order to have clear picture about the athlete coming out of off-season and how preseason training has prepared the athlete for the competitive season. Then, it is recommended that biomarker testing be completed around a single bout of exercise during the competitive season. In order to understand any abnormal responses to acute stress, biomarkers should be tested before and after a bout of exercise during a particularly challenging training week, a performance test, or a bout of exercise after recovery from an injury or after some shift in training. Another recommendation is to test before and multiple times after a major competition or injury. In this case, multiple testing will allow physicians to proper manage recovery and to precisely make a decision regarding the return to play of an athlete (4). The measurement and analysis of biomarkers can be expensive and time consuming, often requiring specific expertise. Furthermore, the data often reveal medical issues that require careful investigation by a physician. Of utmost importance is establishing the baseline values for each athlete of all the biomarkers that will be tested throughout the season. Monitoring the health and performance of athletes via biomarkers is a dynamic process and all the information gathered should be integrated in a context. There is a need to a tailored made approach for each individual, alongside with a holistic view in order to optimize health, wellbeing and performance.

Nutritional assessment
Following a healthy diet enables the athletes' body to function optimally in areas including disease prevention, energy production, immunity physical and mental performance. When nutritional intake is inadequate performance is impaired. So as to develop an individualized nutrition plan or supplementation if necessary (all screened and approved for WADA regulations) extensive tests may be needed and testing various biomarkers is one of them.
Monitoring macronutrients and micronutrients can be of great help in order to prevent or to early discovery of nutritional deficiencies, especially when training volume and intensity changes. Fasting blood glucose measurement is an easy biomarker to measure. In overtraining syndrome (OTS) there have been found lower levels of fasting blood glucose in this way athletes being prone to severe hypoglycemia. Plasma glucose concentration is not affected by exercise if food and drink intakes are correct. Elite power athletes exhibited a relatively lower insulin sensitivity than their endurance counterparts. These data are of importance for the development of chronic diseases in later lifetime: former elite power athletes have a significantly higher relative risk of diabetes and the metabolic syndrome than endurance athletes (6).
Omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), reduce inflammation, muscle soreness, and the perception of pain from exercise. They also play a role in neuromuscular function, nerve conduction velocity, and neuromuscular sensitivity of the acetylcholine receptor. The increased volume and intensity of athletes' training may increase requirements to as 6–8g per day (2:1 ratio of EPA: DHA) (6).

For athletes to support muscle protein synthesis, facilitate training adaptations, and prevent lean muscle mass loss a protein intake of 1.3–2.0 grams per kg body mass and day is recommended. An imbalance between dietary protein intake and dietary protein needs may result in net protein loss in athletes consequently tissue protein breakdown becoming a source of essential amino acids needed to maintain critical body functions. Various biomarkers including total protein, albumin, globulin, blood urea nitrogen (BUN) and amino acid analysis may help athletes' physician to adapt the dietary intake to training. A low protein intake decreases blood proteins. Elevated values of BUN can be due to high protein intake, endogenous protein catabolism, exhaustive exercise, fever, infection, glucocorticoids, state of hydration, hepatic urea synthesis, and renal urea excretion. In the absence of disease, low albumin and high BUN values may suggest an inadequate protein intake (6).
Vitamin D plays an essential role in health including energy production and bone growth to name but a few. Low levels increase the risk of bone injuries such as stress fractures which can have a huge impact on athletic performance. Low levels can also lead to reduced energy, increased inflammation and weaker immune function. Therefore, regardless of the limited literature available in support of a positive effect from vitamin D on performance, obtaining optimal 25(OH)D levels should be a goal for all people. Testing vitamin D levels with the goal of establishing a minimum of 50 ng/dl. is recommended (4).
Adequate iron intake and storage underpins erythropoiesis and the maintenance or increase in total hemoglobin mass with endurance training, particularly at altitude. Athletes have a higher risk of iron deficiency so it is important to ensure their levels remain in the normal range to avoid fatigue. Prolonged iron deficiency can lead to anemia, impaired VO2max, reduced energy efficiency, lower time to exhaustion, lower training volume per day. To a better assessment of an iron deficiency various markers should be tested such as: ferritin, total iron binding capacity, hemoglobin concentration, transferrin, RBC morphology markers.
Accurately measuring magnesium levels can be difficult. Magnesium is found primarily within your cells (intracellular), however, most blood tests for magnesium do not detect a significant deficiency because they measure what is outside the cells (extracellular). Red blood cell (RBC) magnesium test can provide an earlier indication of magnesium deficiency. Deficiencies may lead to muscle weakness, muscle spasms, and altered CK and lactate (4).
Nonetheless, monitoring several vitamins like B group vitamin which are involved in the energy metabolism by modulating the synthesis and degradation of carbohydrate, fat, protein and bioactive compounds, vitamin A and E which act like antioxidants may play an important role in optimizing the athletes' performance.
An assessment of clinical signs, environmental factors, and potential food–drug interactions can complete the task. Overall, the assessment process can help the athlete understand that supplement intake cannot reverse poor food choices and an inadequate diet, while a well-chosen diet can ensure maximal benefit from supplementation.

Muscle status and bone metabolism
According to Lee et al. it is typical for athletes to have elevated CK during training, with reference ranges of 82–1.083 U/L in male and 47–513 U/L in female athletes suggested as athletic norms. Higher increment in CK levels is to be expected in individuals with lower physical fitness, as during initial training periods. Serum CK concentration is typically increased after exercise and an incomplete recovery, that is, a return to basal values, signals the occurrence of trauma or overtraining (4). Also, if CK serum level remain high after detraining a full diagnostic check up with special regards to signs of muscle weakness should be taken into consideration (8).
Muscular injury is accompanied by atraumatic rhabdomyolysis caused by structural damage and protein leakage in the myofibrils, acute inflammatory response, and a decline in muscular strength. For instance, exertional rhabdomyolysis without symptoms can be caused by running a 246 km ultramarathon. CK, LDH, and myoglobin may serve as markers for assessing the degree of muscular injury (8) Myoglobin may leak into circulation during muscle damage (peak 1–3 hours after exercise), and BUN can indicate overall protein synthesis vs. breakdown. Monitoring CK, LDH and myoglobin level to determine an athlete's muscle status during training and recovery will be useful to athletes, coaches, and clinicians. In this way concentration of these three enzymes might be used to monitor return to play (RTP) of athletes with muscular injury.
The free testosterone to cortisol ratio (T:C) is highly-valued by sports scientists as a tool in determining if the body is getting sufficient rest from exercise. A prolonged decrease in T:C ratio is associated with detriments

to performance through increased proteolysis and decreased protein synthesis. A 30% decrease in T:C has been suggested as an indicator of insufficient recovery (4).

Elite athletes have the highest mitochondrial function of all humans. When there is a mitochondrial dysfunction or, simply, mitochondria are not very efficient to clear lactate, it accumulates in the cytosol and then is exported to the blood for further oxidation in most tissues of the body. The acidosis and H+ ions associated with lactate interfere with muscle contraction and can elicit an important decrease in peak contractile force and a decrease in muscle shortening velocity. H+ ions compete with calcium (Ca++) for the Troponin C binding site ions inhibit Ca++ release and uptake from sarcoplasmic reticulum interfering with muscle contraction as well. Undoubtedly, lactate clearance capacity during exercise is a key parameter in athletic performance. Lactate inhibits lipolysis by inhibiting GPR81 receptor in adipose tissue and down regulates cardiolipin activity which regulates both fatty acid transporters CPT-1 and CPT-2 across mitochondria and therefore can interfere with mitochondrial fatty acid transport and oxidation. Therefore, an increased in blood lactate is a surrogate of mitochondrial function (1).

A study with a large cohort of elite athletes (n = 495) with different degrees of cardiovascular demands, where over 750 metabolites were measured found metabolic differences related to source of energy, mechanisms for scavenging oxidative stress, and membrane dynamics between the high cardiovascular demand group compared to their low/moderate counterpart. However more studies are needed to confirm these metabolic differences in independent data set, aiming for discovery of biomarkers for assessing health, performance, and recovery of elite athletes that could be used as early signs of OTS with possible implementations in guiding training programs (5).

When thinking of bone metabolism and phosphate homeostasis calcitriol and parathyroid hormone (PTH) are of great importance. Calcitriol leads to mineralization of bone. In the case of a vitamin D deficiency and a subsequent enteral calcium absorption disorder, a compensatory increase in PTH secretion can stimulate the bone-resorbing osteoclasts. This takes place at the expense of the bone quality and can lead to a deterioration in bone stability. A common reason for disturbed enteral calcium uptake is the (chronic) use of proton pump inhibitors with subsequent hypochlorhydria. In order to be able to assess the balance of the bone metabolism, laboratory-chemical bone formation and bone resorption markers are used. Your measured values reflect the extent of bone formation or degradation (2). Female athletes need special attention when it come to bone metabolism. The linkage between low energy intake, amenorrhea and reduced bone density in women athletes is mediated by endocrinological control, inducing variations in systemic bone formation markers. Since biological bone markers may fluctuate during the menstrual cycle, studies on females should report the time of blood sampling. During training, carboxy-terminal collagen cross-links (CTx), a bone resorption marker, was shown to be less sensitive than amino-terminal cross-linking telopeptide of type I collagen (NTx) and urinary pyridinolines, which were sensitive to anaerobic exercise. Whereas, the bone formation markers, bone alkaline phosphatase (BAP) and osteocalcin (OC) changed after 1 month and 2 months of an exercise program, respectively (7, 10). A laboratory diagnostic assessment carried out regularly (in competitive sports at least twice a year) via Pre-participation Examination and the resulting intervention in the event of abnormal values can help to improve the performance of athletes. A balanced calcium homeostasis should be aimed for through an optimal vitamin D supply and a balanced diet in order to reduce the risk of injuries such as stress fractures.

Could biomarkers discover the early onset of overreaching?

When training continues or when athletes deliberately use a short term period to increase training load, they can experience short-term performance decrement without severe psychological or lasting other negative symptoms. This functional overreaching (FOR) will eventually lead to an improvement in performance after recovery. However, when athletes do not sufficiently respect the balance between training and recovery, non-functional overreaching (extreme OR) can occur. At this stage, the first signs and symptoms of prolonged training distress such as performance decrements, psychological disturbance (decreased vigor, increased fatigue), and hormonal disturbances will occur, and the athletes will need weeks or months to recover. Overtraining syndrome (OTS) is represented by long-term reductions in performance capacity observed over a period of several month (11).

Monitoring the OR and OTS through blood parameters is a fundamental part of training program. Although most of the blood parameters (e.g., blood count, C-reactive protein, erythrocyte sedimentation rate, CK, urea, creatinine, liver enzymes, glucose, ferritin, sodium, and potassium) are not capable of detecting OR or OTS,

they are helpful in providing information on the actual health status of the athlete and therefore useful in the ‘‘exclusion diagnosis.’’
The causes of OTS are various: excessive training (there is no one size fits all), inadequate nutrition, psychological stress. Keeping the right balance between micronutrients and macronutrients is essential to ensure a proper recovery strategy. Having low glycogen deposits due to low carbohydrate diets will impair performance and lead to a decreased anabolic activity as well as to OT. Breaking proteins for gluconeogenesis will also lead to micro tears in the skeletal muscle, which consequently will decrease more the capacity of muscle to store glycogen. When muscle damage is present, cellular components leak into the blood stream. CK, LDH are the most common used biomarkers to assess the muscle tears. Alongside stand troponin I, ALT, AST, cytokines, reactive oxygen species. To early detect muscle damage or even to prevent, metabolomics could provide a better understanding to the anabolic and catabolic states. Moreover, when anabolic processes are decreased, there is a decrease in the production of red blood cells and hemoglobin. Therefore, monitoring hemoglobin during the season or every time a decrease in performance is suspected it is useful to reanalyze the training regime and recovery strategies. There is strong relationship between the hypothalamic-pituitary-adrenal axis and exercise intensity. Furthermore, if the athlete stays a long period of time under stressful physiological conditions, cortisol will increase the catabolic state, whereas the testosterone and growth hormone response will be decreased. Also, training under high cortisol level will impair the immune system function. Special attention should be paid to endurance athletes who are diagnosed with hypothyroidism. Fatigue is the common symptom of chronic overtraining and hypothyroidism; therefore, an increase value of the thyroid stimulating hormone should be assessed very carefully to avoid the situation of an misdiagnosis (1).
A study comparing athletes with OTS and healthy athletes who trained with the same intensity, frequency, and types of exercise showed that neutrophils and testosterone were lower in the OTS group and creatine kinase, lactate, estradiol, total catecholamines, and dopamine were higher in the OTS group (12). A long-term measurement of biochemical parameters in elite soccer players indicated that AST, CK, LDH and creatinine (Cr) levels, when analyzed together, could constitute a useful set of markers in monitoring the recovery period of athletes and diminish the risk of overreaching or injury in soccer players. AST, LDH and Cr seem to be particularly good indicators because of the lower interindividual variability between these parameters in comparison to CK (13).
Training Optimization (TOP) test seems to be more appropriate to distinguish NFO and OTS. The TOP test, which is a two-bout maximal incremental exercise test protocol, was created to detect changes in the typical HPA axis-related hormones (cortisol, ACTH, prolactin[PRL], and human growth hormone [GH]) in response to two exercise tests separated by 4 h. A cutoff value of 200% for the ACTH and PRL response to the second exercise test was shown to be discriminating between NFO and OTS with high sensitivity (>80%). Cortisol and GH responses to the second exercise bout were much less sensitive, as were resting values of these HPA axis hormones (14). Considering female athlete specificities, it should be taken in account that the female menstrual cycle and the use of hormone contraceptives.
The Endocrine and Metabolic Responses on Overtraining Syndrome (EROS) study identified the EROS-COMPLETE set of 20 parameters that combined clinical, basal and stimulated hormones profile, body composition, eating, sleeping and psychological patterns may be a promising tool in early detection of OTS among athletes (12, 15).

New approach when monitoring concussion

There is great interest in identifying objective biomarkers to assist in diagnosis of sport related concussion (SRC), identification of players at risk of delayed recovery, and determination of an athlete’s readiness for RTP after concussion (16). Biomarkers that capture various aspects of the neurometabolic cascade of concussion have been investigated following SRC, including S100 calcium binding protein B (S100B), glialfibrillary acidic protein (GFAP), ubiquitin c-terminal hydrolase-L1 (UCH-L1), alpha-II-spectrin derivatives, and general inflammatory markers on a study on 106 high school and collegiate football players. For example, elevated S100B and UCH-L1 have been reported in multiple samples of athletes with SRC and in emergency department (ED) in patients with traumatic brain injury. GFAP levels were, however, elevated at both acute visits relative to baseline in concussed athletes with loss of consciousness, post-traumatic amnesia, and/or retrograde amnesia suggesting that GFAP may be sensitive specifically to more severe gradients of concussion. There were also reported acute elevation in general markers of inflammation (i.e., IL-6 and IL-1RA) whereas the acute increases in the brain injury markers (i.e., UCH-L1, S100B, and SBDP150) were not associated with symptom duration. Rather, IL-1RA, was significantly associated with the number of days that concussed athletes reported symptoms. The US Food and Drug Administration recently approved the nation's first blood biomarker test for ruling out the need for a Computed Tomography (CT) scan in emergency departments after a suspected brain injury, using UCH-L1 and GFAP.A blood sample collected within 12 hours of injury that is below the cutoff for both UCH-L1 (<327pg/mL) and GFAP (<22 pg/mL) is associated with the absence of intracranial injuries detected with CT (18).

Saliva – an option for biomarkers samples

Saliva represents an increasingly useful auxiliary means of diagnosis due to its relative ease and stress-free collection protocol, especially when blood or urine sampling is not feasible. Its role and connection with several pathological and physiological states enables suitable analysis and predictions (3). Salivary IgA (sIgA) is one of the most investigated markers of immunological involvement in physical activity as it might be related to the risk of upper respiratory tract infection (URTI) Other studies demonstrated that sIgA decreased three days before URTI symptoms appeared. Moreover, a decline in sIgA of approximately 28% occurred three weeks before the onset of an URTI. Recently, it was shown that sIgA significantly decreased just after high-intensity soccer training sessions, and it was totally dependent on volume and intensity of the training sessions performed just before sampling. When collecting T and C hormones from saliva, fundamental care should be taken regarding sampling procedures, especially regarding environment, nutrition, stress, sleep, physical activity, and circadian rhythms. Collection must be performed from unstimulated saliva and after the subjects have rinsed out their mouths with clear water to clean the oral cavity. It also important to highlight that saliva is limited to free steroid hormonal concentrations and may be influenced by the chronotype of the athlete (18).

Mental health of athletes may benefit from testing biomarkers

Depression and anxiety disorders may occur in athletes at least as commonly as the general population. Although not due to the result of an acute injury, overtraining syndrome (OTS) also can threaten the overall mental and physical well-being of an athlete (19). New research finds that blood serum levels of various biomarkers may help predict the onset and progression of clinical depression. The kynurenine (KYN) pathway has garnered some interest. KYN is one of several pathways for metabolizing tryptophan and the possible link between inflammation and depression (20). A reduced activation of mTor due to a reduction of BCAAs could play a crucial and unrecognized factor in the etiology of depression and may provoke depressive symptomatology and lower energy metabolism (21). BCAA, exercise and high protein diets rescue susceptibility to stress by activating the hippocampal BDNF/TRKB signaling in mice exposed to chronic stress (22). A significant biomarkers that increased odds for major depressive disorder (MDD) onset was cortisol. Current studies have a lack of prospective evidence for biomarkers as predictors of onset of MDD and relapse/recurrence (23). The creation of biomarker panels to recognize different serum growth factors, cytokines, hormonal and metabolic markers leading to major depressive disorder's variability and therapeutic responses may therefore be a promising option.

Conclusions

Reference values for blood concentration of biomarkers specifically adapted to physically active people and athletes are lacking which can lead to misclassification or wrong interpretation of the results. It is important to bear in mind that highly trained people can have concentrations of biomarkers which would be pathological in non-trained people, even in routine hematology and biochemistry parameter. Therefore, it is important to adapt reference values as much as possible and to control each subject regularly, in order to establish his/her own reference scale (24).

Regular monitoring of athletes is fundamental to defining the relationship between load and risk of illness in the care of athletes and in research. This includes accurate measurement and monitoring of the sport and non-sport loads of the athletes, and their performance, how they feel (well-being and clinical symptoms) and any illness (25).

The laboratory assessment includes various muscle enzymes, as well as macro and micronutrients, taking into account the individual energy requirements. General reference ranges serve as orientation, whereby an

individual approach with the establishment of individual and possibly sport-specific reference ranges appears sensible for competitive athletes.
A detailed laboratory diagnosis should nowadays be an integral part of Pre-participation Examination of elite athletes. A complete assessment should ideally include dietary evaluation, clinical evaluation, anthropometry and body composition analysis, biochemical testing, functional testing, nutrition-focused clinical examination, and patient history.
Care must be taken to complete collection of the appropriate sample within the constraints of the athlete's training and performance schedule (e.g., fasting blood, collection timing in relation to training or time of day, or random vs. 24hr urine collection), avoid sample contamination (especially for trace minerals), prevent hemolysis of erythrocytes in whole blood, serum, or plasma before processing, and prohibit nutrient breakdown/metabolism before analysis. Some biochemical tests may also be altered by exercise and/or circadian variation and best collected at the same time of day when the athlete is well rested and often fasted. Unfortunately, cut-off- points are not available for all biomarkers (26).
The aim of biomarker innovation in elite sport is to provide more effective support to athletes. Combining technology with greater knowledge generation via enhanced analytical approaches and interpretation capabilities is where value truly lies.
The interest in biomarkers has also spread in the antidoping community with the emerging field of metabolomics. The literature shows that metabolomics could be an appropriate tool to detect performance enhancing hormone abuse by athletes considering the fact the effect of these hormones on athletes' metabolism remain traceable for days even for years. An equivalent of the athlete biological passport based on several metabolic markers - the metabolomics passport could be the future tool in reduce the doping cases (27).
Elite sport serves as a testing ground for biomarker research in order to meet the needs of athletes. Given that many of the imbalances in homeostasis found in athletes undergoing intensive training resemble disease states, there is potential to apply findings to general population. We live in a time of rapid technological change, which provides an opportunity to take advantage of the data that biomarkers provide. It's critical to grasp the complexities of how training, diet, travel, sleep, injury, and psychological well-being connect. If biomarker science will advance our understanding, professional athletes will no longer be the only ones who profit.

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